CALCIUM FOR PREECLAMPSIA PREVENTION (CPEP) TRIAL
REFERENCES & SELECTED ABSTRACTS

NICHD Project

1Z01HD000373 **Calcium Supplementation in Pregnancy to Prevent Preeclampsia**
1ZIAHD000373
Contract Principal Investigator: Richard J. Levine (FY89 – FY10)

Main Study Design

1. Levine RJ, Esterlitz JR, Raymond EG, DerSimonian R, Hauth JC, Ben Curet L, Sibai BM, Catalano PM, Morris CD, Clemens JD, Ewell MG, Friedman SA, Goldenberg RL, Jacobson SL, Joffe GM, Klebanoff MA, Petrulis AS, Rigau-Perez JG. Trial of Calcium for Preeclampsia Prevention (CPEP): rationale, design, and methods. *Control Clin Trials* 1996;17(5):442-69.

Abstract: The results of ten clinical trials suggest that supplemental calcium may prevent preeclampsia. However, methodologic problems and differences in study design limit the acceptance of the results and their relevance to other patient populations. Many of the trials were conducted in countries where, unlike the United States, the usual daily diet contained little calcium. Moreover, none of the trials has reported the outcome of systematic surveillance for urolithiasis, a potential complication of calcium supplementation. In response to the need for a thorough evaluation of the effects of calcium supplementation for the prevention of preeclampsia in the United States, the trial of Calcium for Preeclampsia Prevention (CPEP) was undertaken at five university medical centers. Healthy nulliparous patients were randomly assigned to receive either 2 g supplemental calcium daily (n = 2295) or placebo (n = 2294) in a double-blind study. Study tablets were administered beginning from 13 to 21 completed weeks of gestation and continued until the termination of pregnancy. CPEP employed detailed diagnostic criteria, standardized techniques of measurement, and systematic surveillance for the major study endpoints and for urolithiasis. The nutrient intake of each patient was assessed at randomization and at 32-33 weeks gestation. This report describes the study rationale, design, and methods.

Main Paper

2. Levine RJ, Hauth JC, Curet LB, Sibai BM, Catalano PM, Morris CD, DerSimonian R, Esterlitz JR, Raymond EG, Bild DE, Clemens JD, Cutler JA. Trial of calcium to prevent preeclampsia. *N Engl J Med* 1997;337:69-76.

Abstract: *Background:* Previous trials have suggested that calcium supplementation during pregnancy may reduce the risk of preeclampsia. However, differences in study design and a low dietary calcium intake in the populations studied limit acceptance of the data. *Methods:* We randomly assigned 4589 healthy nulliparous women who were 13 to 21 weeks pregnant to receive daily treatment with either 2 g of elemental calcium or placebo for the remainder of their pregnancies.

Surveillance for preeclampsia was conducted by personnel unaware of treatment-group assignments, using standardized measurements of blood pressure and urinary protein excretion at uniformly scheduled prenatal visits, protocols for monitoring these measurements during the hospitalization for delivery, and reviews of medical records of unscheduled outpatient visits and all hospitalizations. *Results:* Calcium supplementation did not significantly reduce the incidence or severity of preeclampsia or delay its onset. Preeclampsia occurred in 158 of the 2295 women in the calcium group (6.9 percent) and 168 of the 2294 women in the placebo group (7.3 percent) (relative risk, 0.94; 95 percent confidence interval, 0.76 to 1.16). There were no significant differences between the two groups in the prevalence of pregnancy-associated hypertension without preeclampsia (15.3 percent vs. 17.3 percent) or of all hypertensive disorders (22.2 percent vs. 24.6 percent). The mean systolic and diastolic blood pressures during pregnancy were similar in both groups. Calcium did not reduce the numbers of preterm deliveries, small-for-gestational-age births, or fetal and neonatal deaths; nor did it increase urolithiasis during pregnancy. *Conclusions:* Calcium supplementation during pregnancy did not prevent preeclampsia, pregnancy-associated hypertension, or adverse perinatal outcomes in healthy nulliparous women.

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Abstract: *Background:* The cause of preeclampsia remains unclear. Limited data suggest that excess circulating soluble fms-like tyrosine kinase 1 (sFlt-1), which binds placental growth factor (PlGF) and vascular endothelial growth factor (VEGF), may have a pathogenic role. *Methods:* We performed a nested case–control study within the Calcium for Preeclampsia Prevention trial, which involved healthy nulliparous women. Each woman with preeclampsia was matched to one normotensive control. A total of 120 pairs of women were randomly chosen. Serum concentrations of angiogenic factors (total sFlt-1, free PlGF, and free VEGF) were measured throughout pregnancy; there were a total of 655 serum specimens. The data were analyzed cross-sectionally within intervals of gestational age and according to the time before the onset of preeclampsia. *Results:* During the last two months of pregnancy in the normotensive controls, the level of sFlt-1 increased and the level of PlGF decreased. These changes occurred earlier and were more pronounced in the women in whom preeclampsia later developed. The sFlt-1 level increased beginning approximately five weeks before the onset of preeclampsia. At the onset of clinical disease, the mean serum level in the women with preeclampsia was 4382 pg per milliliter, as compared with 1643 pg per milliliter in controls with fetuses of similar gestational age ($P<0.001$). The PlGF levels were significantly lower in the women who later had preeclampsia than in the controls beginning at 13 to 16 weeks of gestation (mean, 90 pg per milliliter vs. 142 pg per milliliter, $P=0.01$), with the greatest difference occurring during the weeks before the onset of preeclampsia, coincident with the increase in the sFlt-1 level. Alterations in the levels of sFlt-1 and free PlGF were greater in women with an earlier onset of preeclampsia and in women in whom preeclampsia was associated with a small-for-gestational-age infant. *Conclusions:* Increased levels of sFlt-1 and reduced levels of PlGF predict the subsequent development of preeclampsia.

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Abstract: *Context:* Preeclampsia may be caused by an imbalance of angiogenic factors. We previously demonstrated that high serum levels of soluble fms-like tyrosine kinase 1 (sFlt1), an antiangiogenic protein, and low levels of placental growth factor (PlGF), a proangiogenic protein, predict subsequent development of preeclampsia. In the absence of glomerular disease leading to proteinuria, sFlt1 is too large a molecule to be filtered into the urine, while PlGF is readily filtered. *Objective:* To test the hypothesis that urinary PlGF is reduced prior to onset of hypertension and proteinuria and that this reduction predicts preeclampsia. *Design, Setting, and Patients*: Nested case-control study within the Calcium for Preeclampsia Prevention trial of healthy nulliparous women enrolled at 5 US university medical centers during 1992-1995. Each woman with preeclampsia was matched to 1 normotensive control by enrollment site, gestational age at collection of the first serum specimen, and sample storage time at −70°C. One hundred twenty pairs of women were randomly chosen for analysis of serum and urine specimens obtained before labor. *Main Outcome Measure:* Cross-sectional urinary PlGF concentrations, before and after normalization for urinary creatinine. *Results:* Among normotensive controls, urinary PlGF increased during the first 2 trimesters, peaked at 29 to 32 weeks, and decreased thereafter. Among cases, before onset of preeclampsia the pattern of urinary PlGF was similar, but levels were significantly reduced beginning at 25 to 28 weeks. There were particularly large differences between controls and cases of preeclampsia with subsequent early onset of the disease or small-for-gestational-age infants. After onset of clinical disease, mean urinary PlGF in women with preeclampsia was 32 pg/mL, compared with 234 pg/mL in controls with fetuses of similar gestational age (*P*<.001). The adjusted odds ratio for the risk of preeclampsia to begin before 37 weeks of gestation for specimens obtained at 21 to 32 weeks, which were in the lowest quartile of control PlGF concentrations (<118 pg/mL), compared with all other quartiles, was 22.5 (95% confidence interval, 7.4-67.8). *Conclusion:* Decreased urinary PlGF at mid gestation is strongly associated with subsequent early development of preeclampsia.

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Abstract: *Background:* Alterations in circulating soluble fms-like tyrosine kinase 1 (sFlt1), an antiangiogenic protein, and placental growth factor (PlGF), a proangiogenic protein, appear to be involved in the pathogenesis of preeclampsia. Since soluble endoglin, another antiangiogenic protein, acts together with sFlt1 to induce a severe preeclampsia-like syndrome in pregnant rats, we examined

whether it is associated with preeclampsia in women. *Methods:* We performed a nested case–control study of healthy nulliparous women within the Calcium for Preeclampsia Prevention trial. The study included all 72 women who had preterm preeclampsia (< 37 weeks), as well as 480 randomly selected women - 120 women with preeclampsia at term (at ≥ 37 weeks), 120 women with gestational hypertension, 120 normotensive women who delivered infants who were small for gestational age, and 120 normotensive controls who delivered infants who were not small for gestational age. *Results:* Circulating soluble endoglin levels increased markedly beginning 2 to 3 months before the onset of preeclampsia. After the onset of clinical disease, the mean serum level in women with preterm preeclampsia was 46.4 ng per milliliter, as compared with 9.8 ng per milliliter in controls (P<0.001). The mean serum level in women with preeclampsia at term was 31.0 ng per milliliter, as compared with 13.3 ng per milliliter in controls (P<0.001). Beginning at 17 weeks through 20 weeks of gestation, soluble endoglin levels were significantly higher in women in whom preterm preeclampsia later developed than in controls (10.2 ng per milliliter vs. 5.8 ng per milliliter, P<0.001), and at 25 through 28 weeks of gestation, the levels were significantly higher in women in whom term preeclampsia developed than in controls (8.5 ng per milliliter vs. 5.9 ng per milliliter, P<0.001). An increased level of soluble endoglin was usually accompanied by an increased ratio of sFlt1:PlGF. The risk of preeclampsia was greatest among women in the highest quartile of the control distributions for both biomarkers but not for either biomarker alone. *Conclusions:* Rising circulating levels of soluble endoglin and ratios of sFlt1:PlGF herald the onset of preeclampsia.

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Abstract: *Objective:* To determine if pre-eclampsia is associated with reduced thyroid function during and after pregnancy. *Design:* Nested case-control study during pregnancy and population based follow-up study after pregnancy. *Setting:* Calcium for Pre-eclampsia Prevention trial of healthy pregnant nulliparous women in the United States during 1992-5, and a Norwegian population based study (Nord-Trondelag Health Study or HUNT-2) during 1995-7 with linkage to the medical birth registry of Norway. *Participants:* All 141 women (cases) in the Calcium for Pre-eclampsia Prevention trial with serum measurements before 21 weeks' gestation (baseline) and after onset of pre-eclampsia (before delivery), 141 normotensive controls with serum measurements at similar gestational ages, and 7121 women in the Nord-Trondelag Health Study whose first birth had occurred in 1967 or later and in whom serum levels of thyroid stimulating hormone had been subsequently measured. *Main outcome measures:* Thyroid function tests and human chorionic gonadotrophin and soluble fms-like tyrosine kinase 1 concentrations in the Calcium for Pre-eclampsia Prevention cohort and odds ratios

for levels of thyroid stimulating hormone above the reference range, according to pre-eclampsia status in singleton pregnancies before the Nord-Trondelag Health Study. *Results:* In predelivery specimens of the Calcium for Pre-eclampsia Prevention cohort after the onset of pre-eclampsia, thyroid stimulating hormone levels increased 2.42 times above baseline compared with a 1.48 times increase in controls. The ratio of the predelivery to baseline ratio of cases to that of the controls was 1.64 (95% confidence interval 1.29 to 2.08). Free triiodothyronine decreased more in the women with pre-eclampsia than in the controls (case ratio to control ratio 0.96, 95% confidence interval 0.92 to 0.99). The predelivery specimens but not baseline samples from women with pre-eclampsia were significantly more likely than those from controls to have concentrations of thyroid stimulating hormone above the reference range (adjusted odds ratio 2.2, 95% confidence interval 1.1 to 4.4). Both in women who developed pre-eclampsia and in normotensive controls the increase in thyroid stimulating hormone concentration between baseline and predelivery specimens was strongly associated with increasing quarters of predelivery soluble fms-like tyrosine kinase 1 (P for trend 0.002 and <0.001, respectively). In the Nord-Trondelag Health Study, women with a history of pre-eclampsia in their first pregnancy were more likely than other women (adjusted odds ratio 1.7, 95% confidence interval 1.1 to 2.5) to have concentrations of thyroid stimulating hormone above the reference range (>3.5 mIU/l). In particular, they were more likely to have high concentrations of thyroid stimulating hormone without thyroid peroxidase antibodies (adjusted odds ratio 2.6, 95% confidence interval 1.3 to 5.0), suggesting hypothyroid function in the absence of an autoimmune process. This association was especially strong (5.8, 1.3 to 25.5) if pre-eclampsia had occurred in both the first and the second pregnancies. *Conclusion:* Increased serum concentration of soluble fms-like tyrosine kinase 1 during pre-eclampsia is associated with subclinical hypothyroidism during pregnancy. Pre-eclampsia may also predispose to reduced thyroid function in later years.